Evaluation of Antibacterial Activities of Some Medicinal Plants from North-West Iran

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Abstract

Objective(s)
Aim of the present study was to screen the antibacterial activities of some medicinal plants extracts traditionally used in Azarbaijan area (Iran).

Materials and Methods
Thirty-six extracts obtained from different parts of ten plants including Tanacetum balsamita L. (Compositae), Muscari caucasicum Baker (Hyacinthaceae), Equisetum arvense L. (Equisetaceae), Achillea millefolium L. (Compositae), Stachys fruticulosa M. Bieb. (Labiatae), Stachys schtschegleevii Sons. ex. Grossh. (Labiatae), Salvia sahendica Boiss & Buhse (Labiatae), Phlomis caucasica Rech. f. (Labiatae), Echium italicum L. (Boraginaceae) and Thalictrum minus L. (Ranunculaceae) from north-west Iran with traditional medicinal use were examined for their antibacterial activities against some Gram-negative strains such as Escherichia coli, Pseudomonas aeruginosa, Salmonella paratyphi and Serratia marcescens, also, Gram-positive strains of Staphylococcus aureus, Micrococcus luteus, Staph. epidermidis, Streptococcus pneumoniae and Bacillus cereus. The filter paper disc diffusion method as well as broth serial dilution technique were applied to screen the antibacterial efficacy of the extracts and determination of minimum inhibitory values.

Results
Results indicated that the majority of tested plant extracts had antibacterial activity at least against one of the selected bacteria, with the exception of Muscari caucasicum. Methanol extract of the aerial part of Thalictrum minus L. (Ranunculaceae) showed the most potent antibacterial activity against Staph. aureus with MIC value of 0.3125 mg/ml.

Conclusion
The results of this study show that most of the studied plants are potentially a good source of antimicrobial agents and support the traditional applications of some of the tested plants.

Keywords: Antibacterial activity, Disc diffusion, Iranian medicinal plants
**Introduction**

Evidence of use of herbal remedies in Iran goes back to the history itself; also, there are lots of scientific documents in this area. For example Rhazes (860-930), a Persian physician, who adopted treatment based on herbs and foods, and avoided synthesized medicine, except for necessity. Ibn Sina (Avicenna, 980–1037) wrote many books on a wide range of topics but he is perhaps most famous for his ‘Laws of Medicine’ which contains sections on the formulation of medicines, general medicine and others that discuss the herbal medicines in details (1-3).

Even today, plant materials continue to play a major role in primary health care as therapeutic remedies in many developing countries (4). However, treatment of infections has been remarkably effective since the discovery of antibacterial drugs, appearance of some resistant pathogens as well as undesirable side effects of certain antibiotics (5-9) have led to the search for new antibacterial agents, in particular from medicinal plants (10-13). The present study was to screen the antibacterial activities of some local medicinal plants extracts traditionally used in Azerbaijian area (Iran); *Tanacetum balsamita* L. (Copmositae), *Muscaria caucasicum* Baker (Hyacinthaceae), *Equisetum arvense* L. (Equisetaceae), *Achillea millefolium* L. (Compositae), *Stachys fruticulosa* M. Bieb. (Labiatae), *Stachys schtschegleevii* Sons. ex Grossh (Labiatae), *Salvia sahendica* Boiss & Buhse (Labiatae), *Phlomis caucasia* Rech. (Labiatae), *Etchium italicum* L. (Boraginaceae) and *Thalictrum minus* L. (Ranunculaceae) against common Gram-negative and positive bacteria. The scientific and local names of the tested plants, parts used and their traditional applications are listed in Table 1.

**Table 1.** Name (scientific and local), family, part used and traditional indications of some medicinal plants from Northwest of Iran.

<table>
<thead>
<tr>
<th>Family</th>
<th>Scientific name</th>
<th>Local name</th>
<th>Part used</th>
<th>Traditional indications</th>
</tr>
</thead>
<tbody>
<tr>
<td>Copmositae</td>
<td><em>Tanacetum balsamita</em> L.</td>
<td>Gia seve</td>
<td>Leaves</td>
<td>Digestive, diuresis, antitusive, analgesic/anti-inflammatory</td>
</tr>
<tr>
<td>Equisetaceae</td>
<td><em>Equisetum arvense</em> L.</td>
<td>Dom asb (Horse tail)</td>
<td>Whole plant</td>
<td>Diuresis, osteoporosis</td>
</tr>
<tr>
<td>Compositae</td>
<td><em>Achillea millefolium</em> L.</td>
<td>Bomadaran</td>
<td>Flowers</td>
<td>Anti-inflammatory, anti-infection</td>
</tr>
<tr>
<td>Labiatae</td>
<td><em>Stachys fruticulosa</em> M. Bieb.</td>
<td>Sonbole gachdust</td>
<td>Aerial parts</td>
<td>Anti-inflammatory</td>
</tr>
<tr>
<td>Labiatae</td>
<td><em>Stachys schtschegleevii</em> Sons. ex Grossh.</td>
<td>Pulk</td>
<td>Aerial parts</td>
<td>Anti-inflammatory</td>
</tr>
<tr>
<td>Labiatae</td>
<td><em>Salvia sahendica</em> Boiss. &amp; Buhse</td>
<td>Maryam gol sahandi</td>
<td>Aerial parts</td>
<td>Anti-inflammatory, anti-infection</td>
</tr>
<tr>
<td>Labiatae</td>
<td><em>Phlomis caucasia</em> Rech.f.</td>
<td>Gush barreh gafgazi</td>
<td>Aerial parts</td>
<td>Analgesic, anti-infection, digestive</td>
</tr>
<tr>
<td>Boraginaceae</td>
<td><em>Etchium italicum</em> L.</td>
<td>Have eluwe</td>
<td>Root and aerial parts</td>
<td>Anti-infection</td>
</tr>
<tr>
<td>Ranunculaceae</td>
<td><em>Thalictrum minus</em> L.</td>
<td>Ghare-ghetarma</td>
<td>Root and aerial parts</td>
<td>Anti-infection</td>
</tr>
<tr>
<td>Hyacinthaceae</td>
<td><em>Muscaria caucasicum</em> Baker</td>
<td>Khazih</td>
<td>Aerial parts</td>
<td>Anti cough</td>
</tr>
</tbody>
</table>

**Materials and Methods**

**Plant materials**

The different parts of plant samples (Table 1) collected and dried at room temperature. The specimens were identified by Dr. H. Nazemiyeh, Mr. Ghahreman and Mrs Eslampanah (Department of Pharmacognosy, Faculty of Pharmacy, Tabriz University of Medical Sciences) and a voucher specimen was kept at the Herbarium of Faculty of Pharmacy, Tabriz University of Medical Sciences.

**Preparation of the extracts**

The dried and grounded plant parts were extracted with different solvents (*n*-hexane, dichloromethane, methanol, chloroform) by maceration for 3 days at room temperature. The extracts combined, filtered and concentrated under reduced pressure at 45 °C till dryness. The residues transferred to small vial and kept at 4 °C before use.
**Antibacterial Activity of Medicinal Plants**

**Bacterial cultures**

Bacterial cultures of Gram-negative species *Escherichia coli* (ATCC 8739), *Pseudomonas aeruginosa* (ATCC 9027), *Salmonella paratyphi* (ATCC 4420) and *Serratia marcescens* (ATCC 33077) as well as Gram-positive strains namely *Staphylococcus aureus* (ATCC 6538), *Micrococcus luteus* (ATCC 10240), *Staphylococcus epidermidis* (ATCC 12228), *Streptococcus pneumoniae* (ATCC 12401) and *Bacillus cereus* (ATCC 9372) were used to evaluate the antimicrobial properties of the selected extracts. The bacterial strains obtained in lyophilized form (purchased from Institute of pasture, Iran) which were cultured in Luria Bertuni agar medium (Scharlau Spain) after suspending them in sterile distilled water. The plates incubated for 24 hrs at 37 °C. Single colony from the plates was transferred into 4 ml fluid of LB medium and incubated over night at 37 °C and 200 rpm in a shaking incubator. The cells harvested by centrifugation at 3000 rpm (Behdad, Iran) for 15 min and at 4 °C. Subsequently, they were washed twice and re-suspended in Ringer solution to provide the turbidity of the 0.5 McFarland standards for disc diffusion method or the concentration range of $10^5-10^6$ CFU/ml for broth dilution method (14).

**Antibacterial assays**

The antimicrobial activity of the tested extracts was monitored using paper disc diffusion method that is a highly recommended method for routine assessment of preliminary antimicrobial screening. This was performed by standard NCCLS methodology, using Mueller- Hinton plates, inoculated with a 0.5 McFarland standard of the selected bacteria. The filter paper discs were impregnated by the extracts (10 µl) and placed on the agar (14). After 48 hrs incubation at 37 °C, inhibition zone diameters read with calipers. The bacteriostatic properties of the active extracts against the most susceptible bacteria namely *Serra. marcescens, Staph. aureus, M. luteus, Staph. epidermidis* and *B. cereus* determined by an evaluation of the Minimum Inhibitory Concentration (MIC). The extracts mixed with Fluid Casein Digest Soya Lecithin Medium (twin pack, Himedia, India) in decreasing concentrations; the tubes inoculated with a 1 ml of inoculum of the tested bacteria (final concentration of $10^5-10^6$ CFU/ml). After 24 hrs of incubation at 37 °C, the tubes were screened for any evidence of bacterial growth. MIC was defined as the lowest concentration of plant extract that completely suppressed the bacterial growth (15). Tubes of DMSO (10%), as solvent for preliminary dissolving or at least homogenization of the extracts and gentamycin sulfate, as positive control, also included.

**Results**

The results for antibacterial activity screening of the selected extracts are shown in Table 2. Among ten plants examined, all of them showed antibacterial effect at least against one of the selected bacterial strains, except *Muscari caucasicum* that showed no activity against any of the tested strains. *Staph. aureus* with 45.5%, *Staph. epidermidis* with 36.5% and *M. luteus* with 33.5% susceptibility were the top three susceptible strains. The most potent effect, related to methanol extract of *Thalictrum minus* that inhibits *Staph. aureus* with inhibition zone diameter of 18.5 mm. MIC values for the active extracts are indicated in Table 3. As shown in Table 3, methanol extract of *Thalictrum minus* with the MIC value of 0.3125 mg/ml against *Staph. aureus* was the most active extracts and the most susceptible bacterium respectively, which confirmed the results of disc diffusion method.
Table 2. Antibacterial activity screening of the selected extracts of the tested medicinal plants from North-west of Iran as inhibition zone diameter (mm).

<table>
<thead>
<tr>
<th>Scientific name</th>
<th>Part of plant</th>
<th>Extracts</th>
<th>Inhibition zone diameter (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Tanacetum balsamita</em> L.</td>
<td>Leaves</td>
<td>Cyclohexane</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Dichloromethane</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Methanol</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Aerial parts</td>
<td>Cyclohexane</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Dichloromethane</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Methanol</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Aqueous</td>
<td>-</td>
</tr>
<tr>
<td><em>Equisetum arvense</em> L.</td>
<td>Whole plant</td>
<td>Ethyl acetate</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Chloroform</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Aqueous</td>
<td>-</td>
</tr>
<tr>
<td><em>Achillea millefolium</em> L.</td>
<td>Flower</td>
<td>Ethyl acetate</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Chloroform</td>
<td>-</td>
</tr>
<tr>
<td><em>Stachys fruticulosa</em> M. Bieb.</td>
<td>Aerial parts</td>
<td>Dichloromethane</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Methanol</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Cyclohexane</td>
<td>-</td>
</tr>
<tr>
<td><em>Stachys schtschegleevii</em> Sons. ex Grossh.</td>
<td>Aerial parts</td>
<td>Dichloromethane</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Methanol</td>
<td>-</td>
</tr>
<tr>
<td><em>Salvia sahendica</em> Boiss. &amp; Buhse</td>
<td>Aerial parts</td>
<td>Dichloromethane</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Methanol</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Cyclohexane</td>
<td>-</td>
</tr>
<tr>
<td><em>Phlomis caucasia</em> Rech. f.</td>
<td>Aerial parts</td>
<td>Dichloromethane</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Methanol</td>
<td>-</td>
</tr>
</tbody>
</table>


Table 3. Minimum inhibitory concentration (MIC) of the most active plants extracts and antibiotic (Gentamicin sulfate) against some of the bacteria.

<table>
<thead>
<tr>
<th>Scientific name</th>
<th>Extract</th>
<th>MIC value (mg/ml) against:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td><em>Serra. marcescens</em></td>
</tr>
<tr>
<td><em>Tanacetum balsamita</em> L.</td>
<td>Cyclohexane</td>
<td>1.25</td>
</tr>
<tr>
<td></td>
<td>Dichloromethane</td>
<td>1.25</td>
</tr>
<tr>
<td></td>
<td>Methanol</td>
<td>1.25</td>
</tr>
<tr>
<td><em>Equisetum arvense</em> L.</td>
<td>Dichloromethane</td>
<td>2.5</td>
</tr>
<tr>
<td></td>
<td>Methanol</td>
<td>2.5</td>
</tr>
<tr>
<td><em>Achillea millefolium</em> L.</td>
<td>Dichloromethane</td>
<td>2.5</td>
</tr>
<tr>
<td></td>
<td>Methanol</td>
<td>2.5</td>
</tr>
<tr>
<td></td>
<td>Aqueous</td>
<td>2.5</td>
</tr>
<tr>
<td><em>Stachys fruticulosa</em> M. Bieb.</td>
<td>Methanol</td>
<td>2.5</td>
</tr>
<tr>
<td><em>Stachys schtschegleevii</em> Sons. ex Grossh.</td>
<td>Methanol</td>
<td>2.5</td>
</tr>
<tr>
<td><em>Salvia sahendica</em> Boiss. &amp; Buhse</td>
<td>Methanol</td>
<td>2.5</td>
</tr>
<tr>
<td><em>Phlomis caucasia</em> Rech. f.</td>
<td>Methanol</td>
<td>2.5</td>
</tr>
<tr>
<td><em>Etchium italicum</em> L. (root)</td>
<td>Cyclohexane</td>
<td>2.5</td>
</tr>
<tr>
<td></td>
<td>Dichloromethane</td>
<td>2.5</td>
</tr>
<tr>
<td><em>Thalictrum minus</em> L. (root)</td>
<td>Cyclohexane</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Dichloromethane</td>
<td>-</td>
</tr>
<tr>
<td><em>Thalictrum minus</em> L. (aerial parts)</td>
<td>Cyclohexane</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Dichloromethane</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Methanol</td>
<td>2.5</td>
</tr>
<tr>
<td>Gentamicin sulfate</td>
<td>0.8</td>
<td>1.2</td>
</tr>
</tbody>
</table>
Antibacterial Activity of Medicinal Plants

Discussion
The present study was conducted to investigate the in vitro antimicrobial activity of some local medicinal plants used by people of North-west of Iran to evaluate the scientific base of their application. However, the plants differ significantly in their activity against test microorganisms, nearly all of the extracts evaluated, with the exception of Muscaria a各项工作um, were active against at least one of the Gram-positive strains. The most susceptible bacteria group was the Gram positive strains, among them Staphylococcus aureus and Staphylococcus epidermidis that cause serious infections in human and other animals including superficial skin lesion, localized abscesses, and food poisoning (16) were in the first positions. Due to the importance of Staphylococcus aureus in the above mentioned conditions, plants such as Thalictrum minus that showed high activity against Staphylococcus aureus is of great importance.

Gram-negative strains except Serratia marcescens almost showed no susceptibility, this finding is in good agreement with results obtained by several researches. Duarte and co-workers (2005), Skaltsa et al (1999, 2003) as well as Khanavi and co-workers that evaluated the antimicrobial effect of methanol and essential oils of different strains of the Stachys genus showed similar results (17-20). This could be due to several possible reasons; one is the presence of a double membrane surrounding each bacterial cells. This outer membrane excludes certain drugs and antibiotics from penetrating the cell, partially accounting for why Gram-negative bacteria are generally more resistant to antibiotics than other Gram-positive bacteria (21-22).

Three of the least susceptible bacteria were Escherichia coli, Strep. pneumonia and P. aeruginosa. The latter is one of the most commonly-isolated nosocomial pathogen accounting for a significant percentage of hospital-acquired infections. Due to multi-resistance feature of P. aeruginosa, finding an effective antimicrobial agent against this microorganism is a difficult task, resulting in the increasing trend of nosocomial infections in hospitals and health care centers.

The most potent antibacterial effect related to methanol extract of Thalictrum minus that inhibit Staphylococcus aureus with inhibition zone diameter of 18.5 mm. MIC values which were determined only for the active extracts, are indicated in Table 3. Methanol extract of Thalictrum minus with the MIC value of 0.3125 mg/ml against Staphylococcus aureus was the most active extract and the most susceptible bacterium respectively, which confirm the results of disc diffusion method.

Although the nature and number of active antibacterial components involved in each extract are not clear, but the broad spectra of activity of several plants extracts such as Thalictrum minus, Salvia sahendica, Achillea millefolium and Echinum italicum, especially methanol extracts, however, are promising and the isolation of active constituents of each extract can be the subject of next researches.

Conclusion
In conclusion, some of the people from North-west of Iran employ medicinal plants for their health problems like inflammation, infections, and urinary tract disorders. The results of this study have shown that most of the studied plants are potentially a good source of antimicrobial agents and support the traditional medicinal application of some of the tested plants.

Acknowledgement
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References
Farzaneh Lotfipour et al